

# A Study of Non-Syndromic Hypodontia in a High Risk Population

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## **Abstract**

### **Aim**

To investigate autosomal recessively inherited forms of severe hypodontia in a consanguineous population.

### **Background**

Severe hypodontia maybe associated with different types of inheritance, including autosomal recessive. The frequency of such disorders tends to increase in population where intermarriage between blood relatives is common. A cohort of young individuals with severe hypodontia and parental consanguinity was studied to determine if there were features of an autosomal recessively inherited disorder.

### **Materials and Methods**

10 families with a known history of consanguinity were recruited and interviewed regarding their hypodontia status. In the West Midlands, families that originated from a Pakistani or Bangladeshi background have a high frequency of parental consanguinity so a search was undertaken through the Birmingham Dental Hospital database in an attempt to identify patients with a Muslim family name for further study. A detailed family pedigree was drawn for selected subjects by interviewing the parents. Living affected subjects were examined and peripheral blood samples were collected at Birmingham Dental Hospital with informed consent. Dental examination revealed severe hypodontia i.e. six or more missing teeth in one affected individual from each family. Molecular genetic analysis using autozygosity mapping techniques were used to identify autosomal recessive

gene mutations causing severe hypodontia.

## **Results**

The subjects had similar phenotypes. Dental anomalies such as microdontia, taurodont molars, hypoplasia of the enamel, infraocclusion, shortened dental roots and ectopic teeth were recorded, as well as the typical skeletal/facial traits expected in a hypodontia population. The pattern of dental agenesis demonstrated heterogeneity amongst the probands and appeared to be more varied than that described previously, with the involvement of atypical teeth not often found to be congenitally missing.

## **Conclusion**

Four out of the ten families had affected members of the family, which supports a genetic basis for the severe hypodontia seen in the families. These observations provide a basis for molecular genetic studies to define the exact genetic cause of the disorder.

## **Acknowledgements**

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## **Dedication**

I dedicate this research to my brothers Rishi and Pardeep and my mother and father, without whose support this project would not be possible.

## Key

Note nomenclature used in text; upper case refers to humans and lower case refers to mouse.

Italicized nomenclature refers to genes. (see example):

MSX1 = Human Msh Homeobox 1 protein

*MSX1* = Human Msh Homeox 1 gene

msx1 = Mouse Msh Homeobox 1 protein

*msx1* = Mouse Msh Homeobox 1 gene

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## 1.0 Introduction

Hypodontia describes the congenital absence of teeth. Other terms describing a reduction in the number of teeth include oligodontia, anodontia, aplasia of the teeth, congenitally missing teeth, absence of teeth and agenesis of teeth and lack of teeth (Adeboye *et al.*, 2006; Cobourne and Sharpe, 2013).

Environmental and genetic factors contribute to the overall process of tooth development. Three major genes MSX1, PAX9, AXIN2, play a part in the aetiology of non-syndromic hypodontia and their study has contributed much to the understanding of the genetic aspects of hypodontia (Cobourne, 2007). However family studies suggest that MSX1 and PAX9 mutations are not consistently found in families with hypodontia, indicating that other genes may be responsible for this anomaly (Song *et al.*, 2009). This study is directed at identifying a novel gene responsible for autosomal recessive inherited hypodontia in order to facilitate understanding of dental agenesis.

## **1.1 Definitions and Classification**

The term hypodontia is associated with the absence of a few (1- 6 excluding the third molars) missing teeth whereas oligodontia describes a large number of missing teeth (more than 6 teeth excluding the third molars) (Arte and Pirinen, 2004; Polder, 2004). Anodontia signifies the total absence of teeth. Although the terms selective tooth agenesis is emerging in the more contemporary literature the former terms are considered more accurate and hence will be used throughout the course of this report (Cobourne and Sharpe, 2013).

Further diagnostic classifications (Goodman *et al.*, 1994; Dhanrajani, 2002; Nunn et al., 2003; Jones, 2009) describe the severity of hypodontia arbitrarily as:

Mild: 1-2 missing teeth

Moderate: 3-5 missing teeth

Severe: 6 or more missing teeth

Partial anodontia is no longer used in the literature (Jones, 2009).

<b>Classification of inherited tooth loss</b>	
<b>Non syndromic (familial)</b>	Hypodontia
	Oligodontia
	Anodontia
<b>Syndromic</b>	Hypodontia
	Oligodontia
	Anodontia

**Table 1 .1 Courtesy of Cobourne, 2007**

When prioritising the need for treatment in secondary care services the number of missing teeth is factored into the severity of need for treatment through the scoring system of the index of orthodontic treatment need in the dental health component (IOTN). Absence of more than a single tooth in a quadrant scores 5 whilst cases with fewer missing teeth score 4 (Hobkirk *et al.*, 2011). The fact that hypodontia indicates high treatment need is reflected by other indices (Otuyemi and Jones, 1995; Shelton *et al.*, 2008).

A greater emphasis on oral health in the modern era has meant individuals suffering from hypodontia are requesting treatment for their disorder, which has obvious implications for the health services (Hobkirk *et al.*, 2011).

Hypodontia can contribute to the severity of an overlying malocclusion and negatively impacts on an individual's psychosocial well being (Gill *et al.*, 2008). Research has looked into the impact of medical conditions on the quality of life. The World Health Organisation Quality of Life (WHOQoL) group defined QoL as the "individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns'. QoL is complex and multidimensional and has been shown to be related to OHRQoL (Taylor *et al.*, 2009). Oral health related quality of life encompasses different domains, including survival of the dentition, function and aesthetics (Cunningham and Hunt, 2001).

There are financial implications to patients and the healthcare service and can potentially contribute to the overall costs over a patient's lifetime. The dental implications include;

- Psycho-social implications
- Medical implications
- Functional implications
- Educational implications

- Financial implications.

Comprehensive treatment can be complex as well as expensive which places a huge burden on the National Health Service resources. Table 1.2 summarises the complex management of the disorder at various stages throughout an individual's overall management.

Age	Treatment	Comments
< 6 years Deciduous dentition	Removable dentures for psychological reasons	Regular adjustments during growth Retention
7-12 years  Mixed dentition	Composite build-ups to improve aesthetics  of microdont permanent teeth or worn deciduous teeth.  Removable dentures.  Consider interceptive extractions to guide eruption.  Simple orthodontic treatment for space redistribution.	Problems may include palatal maxillary canines and infraocclusion.  e.g. a diastema that cannot be closed restoratively.  Long-term retention will be required.
>12years  Permanent dentition	Orthodontic treatment  Resin-bonded bridges following orthodontics for tooth replacement.  Composite build-ups of microdont or hypoplastic teeth.  Overdentures (severe hypodontia)	Pontics can be placed on the fixed appliance and the retainer following orthodontics as a temporary measure.  Other methods of tooth replacement include maintaining the deciduous predecessor, dentures, fixed bridges and transplantation.  Disguising intense hypoplastic patches can be difficult.  Abutments help maintain alveolar bone, improve retention and stability and provide proprioception.
16-20 years	Single tooth implants or implant fixed bridges or implant-retained overdentures.  Orthodontics in combination with orthognathic surgery.	Placed when the majority of growth is complete.  Tends to be earlier in females (17 years) than males (18 years). Bone augmentation procedures may be required before implant placement.  For patients with severe skeletal discrepancies.

**Table 1.2 adapted from “Counselling patients with hypodontia” Gill *et al.*, 2008**

The implications of on going dental maintenance for individuals with prosthetic replacements must be factored into the financial costs not only for the patients but also for the service provider (Forgie *et al.*, 2005; Thind *et al.*, 2005; Hobkirk, 2006). Since a number of specialities are involved in the treatment pathway of a hypodontia patient it is important to collate data for the planning and allocation of healthcare resources at regional and national levels (Hobkirk *et al.*, 2011).

## **1.2 Epidemiology**

Hypodontia is relatively uncommon in the primary dentition with a prevalence of only 0.1-0.9% affecting males and females equally (Grahnen and Granath, 1961; Jarvinen and Lehtinen, 1981; Carvalho *et al.*, 1998; Dhanrajani, 2002; Nunn *et al.*, 2003). There is a strong association between hypodontia affecting the primary and permanent dentition. The general dental practitioner is in an ideal position to identify the diagnosis and thus arrange referrals for further investigations and family counselling if necessary (Arte and Pirinen, 2004).

The prevalence of hypodontia in the permanent dentition often varies as figures are reported from studies with relatively small sample sizes. A meta-analysis reported that the prevalence of dental agenesis was estimated between 0.3-36.75% and attempted to establish the prevalence of dental agenesis by looking at different ethnic populations (Table 1.3)



	<b>Males</b>	<b>Females</b>	<b>Total</b>
<b>Europe (White)</b>	4.6 (4.5–4.8)	6.3 (6.1–6.5)	5.5 (5.3–5.6)
<b>North America (White)</b>	3.2 (2.9–3.5)	4.6 (4.2–4.9)	3.9 (3.7–4.1)
<b>North America (African American)</b>	3.2 (2.2–4.1)	4.6 (3.5–5.8)	3.9 (3.1–4.6)
<b>Australia</b>	5.5 (4.4–6.6)	7.6 (6.0–9.2)	6.3 (5.4–7.2)
<b>Saudi Arabia (White)</b>	2.7 (2.0–3.4)	2.2 (1.2–3.1)	2.5 (1.9–3.1)
<b>Chinese Mongoloid</b>	6.1 (4.0–8.1)	7.7 (5.4–10.0)	6.9 (5.3–8.4)

**Table 1.3 Adapted from A meta-analysis of dental agenesis of permanent teeth Polder *et al.*, 2004**

An inadequate sample size precluded the use of the African American, Chinese and Arabic population and although they were looked they were not included in the overall meta-analysis (Polder 2004). A meta-analysis has evaluated the prevalence of tooth agenesis by considering 10 studies with a sample of over 48,000 individuals. Mandibular second premolars (3.0%), followed by maxillary lateral incisors (1.7%) and maxillary second premolar (1.5%) were found to be the most frequently missing teeth. Amongst the least common missing teeth included the mandibular canine (0.02%) the mandibular first molar (0.01%) and the maxillary central incisor (0.005%) (Polder *et al.*, 2004).

The frequency of missing teeth within a sample of hypodontia patients, from 24 studies and a total of approximately 11,500 absent teeth found a similar sequence as that described above; mandibular second premolar (41%) > maxillary lateral incisor (22.9%) > maxillary second premolar (21.2%) > mandibular lateral incisor (2.5%) (Hobkirk *et al.*, 2011). The remaining teeth were within the range of 0.2-1.4%, emphasising previous literature suggesting that the absence of maxillary central incisors, canines and first molars is rare and occurs more often than not in patients suffering from severe hypodontia. Most patients with congenitally absent teeth (83%) had only 1-2 missing teeth. Patients with 3-5 missing teeth represented 14.4% of the group, while severe hypodontia with six or more missing teeth were present in 2.6% of the sample (Hobkirk *et al.*, 2011). This equated to a population prevalence of 0.14%. Females were more affected than males by a factor of 1.37.

Most authors reported a small non-significant sex-difference; however other authors reported a significantly larger frequency in females than in males (Rose, 1966). The cohort of patients studied, consisted of orthodontic patients so despite having an adequate sample size it could not be regarded as representative for the general population (Polder, 2004)

### 1.3 Overview of normal tooth development

The interaction between epithelial and mesenchymal tissues is essential for the correct development of the human tooth, which is characterised by three overlapping stages: initiation, morphogenesis and histogenesis (Berkowitz *et al.*, 2002). The initiation phase represents the stage of development where the future tooth germ is laid seen by invaginations along the oral epithelium called the dental lamina. Morphogenesis determines the eventual shape of the tooth and is characterised by increased cell number and organisation. The differentiation of cells results in the formation of specialised dental tissues, which marks the final stage of histogenesis.

#### 1.3.1 Theories on abnormal tooth development

A disruption in tooth development can result in a variety of clinical features, such as variations in the pattern of tooth agenesis, morphology and tooth size dimensions.

Both genetic and environmental factors have been implicated in the aetiology of tooth agenesis. Exposure to radiation therapy, chemotherapeutic agents and the use of thalidomide drugs during pregnancy can be the main environmental factors responsible (Adeboye *et al.*, 2006; De Coster *et al.*, 2009). In most familial cases of hypodontia the mode of inheritance is autosomal dominant although there have been reports of autosomal recessive and X-linked traits (Cobourne, 2007).

Theories about tooth agenesis have evolved with time over the last few decades. Explanations as to why certain teeth fail to form more than others is based on a theory that the mammalian dentition can be divided into regions which correspond to incisors, canines' premolars and molars. These regions have been described as "morphological fields" and each field has a stable or "key" tooth. This would suggest that most mesial tooth in each series is the most genetically stable whereas the more distal tooth in the series is less genetically stable and therefore more prone to being missing.

This has also been described as Butler's field theory for the evolutionary development of mammalian teeth (Vastardis, 2000; Hobkirk *et al.*, 2011).

This led to the concept of stable and unstable elements of the dentition (Bailit, 1975). This theory was further supported by Bolk's Theory of Terminal Reduction, which described the reduction of the distal element of tooth groups. This resulted in the more frequent absence of second premolars lateral incisors and third molars (de Beer, 1951; Rozsa *et al.*, 2009).

In a sample of 3557 human subjects the most posterior tooth of a series was reported missing most frequently. The teeth most often missing were considered potentially degenerate structures with little or no functional use for modern man with eventual loss altogether (Clayton, 1956).

An anatomic model has been used to replace the previously described evolutionary model in order to explain why certain teeth are more susceptible to tooth agenesis (Svinhufvud *et al.*, 1988). It considered "embryonic fusions": regions of tooth development, which were more vulnerable to environmental and genetic factors. The most common missing tooth in the permanent dentition, the maxillary lateral incisor exemplifies this model since it develops in the area of the "embryonic fusion" between the lateral maxillary and medial nasal processes. The corresponding scenario in the mandible includes the second premolar, which develops in a region of the oral epithelium corresponding to the distal end of the primary dental lamina. The mandibular symphysis corresponds to the region where the two lower central incisors eventually develop and is also susceptible to agenesis as it is the site of fusion during the early development of the mandible (Stritzel *et al.*, 1990).

Sites of tooth agenesis have also been linked to time of innervation in the developing foetus (Kjaer, 1997).

## 1.4 Hypodontia

Developments of the understanding of genetics have led to new theories to explain tooth agenesis.

### 1.4.1 Environmental Factors

Both invasive and non-invasive environmental factors can cause tooth agenesis and they may act additively or independently (Riesenfeld, 1970). Jaw fractures and tooth extraction can affect tooth development and lead to hypodontia (Grahnen, 1956; Schalk-van der Weide *et al.*, 1992; Arte, 2001).

Both chemotherapy and radiotherapy affect tooth development (Maguire *et al.*, 1987; Nasman *et al.*, 1997). The anti-emetic thalidomide caused congenitally missing teeth in children whose mother took the drug in pregnancy (Schubel *et al.*, 1965; Spiers, 1965; Axrup *et al.*, 1966). No definite aetiological link has been found between hypodontia and systemic disease, endocrine disturbances or ectodermal dysplasia (Grahnen, 1956; Arte, 2001).

### 1.4.2 Genetic factors

Hypodontia is more often seen in individuals with a family history of missing teeth than in the general population. This highlights the importance of the genetic component of the disease (Pemberton, 2005). There is often a single gene defect, which is transmitted in most cases as an autosomal dominant trait with variable expressivity and incomplete penetrance (Burzynski and Escoba, 1983; Svinhufud *et al.*, 1988).

The genetic component of the disease is further confirmed by looking at monozygotic twins and triplets (Gravely and Johnson, 1971). The mode of transmission is autosomal dominant with a reported rate of incomplete penetrance of up to 86%, which confirms the strength of heritability (Arte and Pirinen, 2004)

In comparison with the general population, there is a higher frequency of reported association between hypodontia and peg shaped lateral incisors in 1st and 2nd degree relatives (Arte and Pirinen, 2004). A polygenic model, which involves the interaction between genetic and environmental factors, has been suggested as a possible cause of this dental anomaly (Suarez and Spence, 1974; Bailit, 1975). A large Swedish study, which looked at 171 affected subjects, found a strong genetic component (Grahnen, 1956).

Although autosomal dominant transmission appears to be the most common mode of inheritance responsible for familial hypodontia and oligodontia (Grahnen, 1956; Burzynski and Escobar, 1983) sex-linked, autosomal recessive and multifactorial models of inheritance have also been suggested (Suarez and Spence, 1974; Chosack *et al.*, 1975; Brook, 1984; Peck *et al.*, 1994). Two separate family studies support an autosomal recessive mode of inheritance. In Pakistan hypodontia was detected along with other dental anomalies as an autosomal recessive trait (Ahmad *et al.*, 1998) and in Finland 31 family pedigrees were studied and in two families a common ancestor was determined of the parents of the affected individuals (Pirinen, 2001).

A multifactorial model has attempted to explain the association between hypodontia and microdontia (Lyngstadaas *et al.*, 1996) based on the theory of tooth size limit, whereby a reduction in the size of the tooth may reach a certain limit or “threshold” causing a failure of the tooth germ to develop (Brook, 1984). This phenomenon has been demonstrated by studies in which the prevalence of peg shaped laterals incisors was greater (5.5%) than the 1.5% prevalence found in the general population (Grahnen, 1956; Alvesalo and Portin, 1969). Anomalies in tooth number is often associated with other aberrant features of dental development such as small tooth size, ectopia enamel defects and transpositions (Cobourne, 2007)



#### 1.4.3 Non-syndromic hypodontia

Non-syndromic hypodontia is more frequently seen in the secondary dentition. Anodontia is rare in the absence of any genetic disease, while oligodontia is only seen in 0.25% within European populations (Cobourne, 2007). Incisor-premolar hypodontia is more common affecting approximately 8% of the population with the involvement of anything from one to twelve missing teeth (Nieminen *et al.*, 1995). Certain teeth fail to develop more often than others and the canine teeth, first molars and second molars are missing only in severe forms of oligodontia (Hobkirk *et al.*, 2011).

Non-syndromic hypodontia can be classified as either sporadic or familial and can be inherited in an autosomal dominant, recessive or X-linked mode. Dominant mutations in *MSX1*, *PAX9* and *AXIN2* have been found in families with non-syndromic hypodontia (Vastardis *et al.*, 1996; Stockton *et al.*, 2000; Lammi *et al.*, 2004).



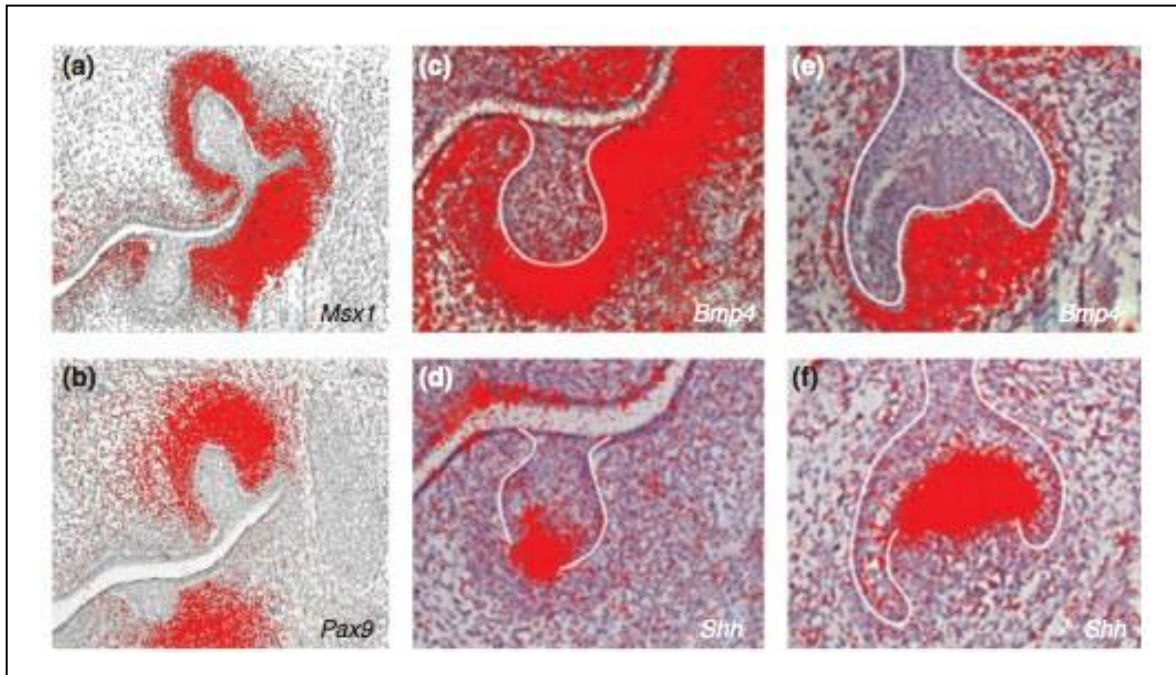


Figure 1.4.3.1 by kind permission of Cobourne and Sharpe, 2013, Personal communication

This diagram demonstrates the importance of MSX1 and PAX9 during early tooth development (a, b). Mice lacking MSX1 and PAX9 demonstrate a failure in normal tooth development (Cobourne and Sharpe, 2013).

It is possible that other genes may be responsible for this anomaly as mutations in these genes could only account for a few affected individuals (Scarel *et al.*, 2000; Frazier-Bowers *et al.*, 2002). Mutations associated with the *EDA* gene could be responsible for non-syndromic oligodontia in affected males with reports of affected individuals in a Chinese family with X-linked hypodontia (Song *et al.*, 2009). Another mutation in the *EDA* gene was also detected in affected members of an Indian family with X-linked hypodontia (Tarpey *et al.*, 2007). Studies suggest that the *FGFR1* gene responsible for regulating osteoblast and chondroblast differentiation may be implicated for the absence of premolars in families with non-syndromic hypodontia (Song *et al.*, 2009).

Mutations associated with the *TGFA* gene, whose expression is important in the formation of the palate, maybe be responsible for isolated incisor hypodontia.

These signalling molecules play an important role in controlling the final pattern for tooth number, cusp position and number (De Coster, 2009; Galluccio *et al.*, 2012).

#### 1.4.4 Syndromic Causes of Hypodontia

Over 60 syndromes are categorised on the database On-line Mendelian Inheritance in Man (OMIM) as being associated with hypodontia (Vastardis, 2000). Hypodontia features in syndromes such as Downs, Klinefelter and Wolf-Hirschhorn, Reiger and Ellis-van Creveld syndromes (De Coster *et al.*, 2009).

Mutations in protein Pitx2 are associated with Axenfeld-Reiger syndrome; an autosomal dominant condition is often associated with the triad of ocular, dental and umbilical disorders (Tumer and Holm, 2009). Oro-facial digital disorders are associated with mutations associated of the tumour protein p63, along with digital disorders, facial clefts, cleft lip and palate and ectodermal dysplasia

(Hobkirk *et al.*, 2011). Mutations associated with MSX1 have also been associated with isolated cleft lip and palate (Jumlongras *et al.*, 2001).

There are over 190 different subtypes of ectodermal dysplasia the commonest of which is hypohidrotic ectodermal dysplasia. A site on the X chromosome encodes the protein ectodysplasin A (Eda), which has been shown to be associated in non-syndromic isolated X-linked hypodontia (Hobkirk *et al.*, 2011). Defects in the Eda pathway result in disorders of tooth numbers, size and morphology. This strengthens the argument that the association of hypodontia, microdontia and morphology of teeth may share a common mechanism.

Over 200 syndromes demonstrate concomitant cleft lip/cleft palate. There is a 50% prevalence of hypodontia with mandibular teeth commonly affected in Pierre-Robin syndrome with associated cleft palate micrognathia and glossoptosis, The classical features of Van der Woude syndrome comprise pitting of the lower lip mucosa and hypodontia in approximately 70% of cases. The *IRF6* gene, which is active in the developing craniofacial region including the medial surfaces of the palatal selves and the developing tooth buds, appears to be responsible. Mutations in these regions have been reported in individuals with Van der Woude Syndrome (De Coster *et al.*, 2009).

#### 1.4.5 Genes involved in hypodontia

Familial hypodontia may show an autosomal dominant, autosomal recessive or an X-linked mode of inheritance (Cobourne, 2007). Genes are important in the aetiology of isolated, non-syndromic hypodontia and the transcription factors encoded by MSX1 and PAX9 have been implicated. Other genes associated with hypodontia are the *IRF6*, *TGFA*, *FGR1*, *AXIN2*, *EDA*, *EDAR*, *EDARADD* and *WNT10A* (Galluccio *et al.*, 2012; Cobourne and Sharpe, 2013). Incomplete penetrance means that some patients with a gene mutation have hypodontia / oligodontia which does not affect other

individuals with the same gene mutation (Ruf *et al.*, 2013). Carriers in the same family exhibit significant variability in the number of teeth, their location and symmetry within the dental arch. A better understanding of the genetics may contribute to a better diagnosis of some multiple congenital (anomaly) syndromes involving teeth and improved insight in the genotype – phenotype relationship. Morphological tooth traits, tooth dimension and agenesis patterns may serve as biomarkers of genetic disorder (Kapadia and Mues, 2007).

Tooth development is dependent on genes that encode transcription factors and signal molecules during early tooth formation (Zhao *et al.*, 2007).

MSX1 is a transcription factors- necessary for normal craniofacial development. It acts repetitively during tooth development, which arrests at the bud stage in mice lacking a functional MSX1 gene. It has been associated with familial oligodontia and syndromic hypodontia (Cohen, 2000).

MSX1 is located on chromosome 4 (4q16.1) and appears to be the gene responsible for some forms of congenital teeth agenesis and five mutations have been identified (Kim, 2006). A mutation of MSX1, due to protein defects causes absence molars and premolars and for cleft lip and palate (Lidral and Riesing 2002).

PAX9 belongs to the paired box domain gene family that is named according to the presence of a DNA-binding “paired” domain and is located on chromosome 14 at 14q12-13q. It regulates the pathway and differentiation of stem cells during the bud, cap and bell stages of tooth development (Thesleff, 2003). In mice PAX9 regulates mesenchymal cells during the expression of MSX1 (Peters *et al.*, 1998).

Mutations in PAX9 are often responsible for the agenesis of six more missing posterior dental units resulting in oligodontia (Nieminen *et al.*, 2001; Frazier-Bowers *et al.*, 2002; Das 2003). However

recent studies report a different phenotype in which mutations of PAX9 are associated with missing premolars, canine's incisors and microdontia; canines are rarely missing (Lammi *et al.*, 2003). Most of the mutations associated with PAX9 have been heterogeneous and reports suggest that lack of the resultant gene product produced by PAX9 can result in severe hypodontia (Das *et al.*, 2002). Mutations associated with AXIN2 have been implicated in a more varied pattern of tooth agenesis with an increased number of missing units compared to MSX1 and PAX9. Disorders involving the MSX1 and PAX9 genes include missing molars; premolars lower incisors and upper lateral incisors (Cobourne, 2007). Some individuals with tooth agenesis have no identified mutations in either the PAX9, MSX1 or AXIN2 genes. There are still many individuals with tooth agenesis but having no identified mutation in either the PAX9, MSX1 or AXIN2 gene (Arte, 2001; Kapadia *et al.*, 2007).

#### 1.4.6 Molecular mechanisms involved in odontogenesis

Animal studies have revealed the role of genes in tooth development (Cobourne, 2007). These are shown diagrammatically in Figure 1.4.6.1 (Brook, 1984). Genes act on the epithelial and mesenchymal tissues and the important ones include (Galluccio *et al.*, 2012):

##### **1) Bone morphogenetic protein (Bmp)**

These growth factors stimulate the transcription factors MSX1, MSX2, the EGR1 (early growth-response), and the LEF-1 transcription factor.

##### **2) Fibroblast growth factor (Fgf)**

The FGF family (FGF1–19) acts during the early phases of tooth development and control the formation of ameloblasts and odontoblasts.

##### **3) Tumour necrosis factor (TNF)**

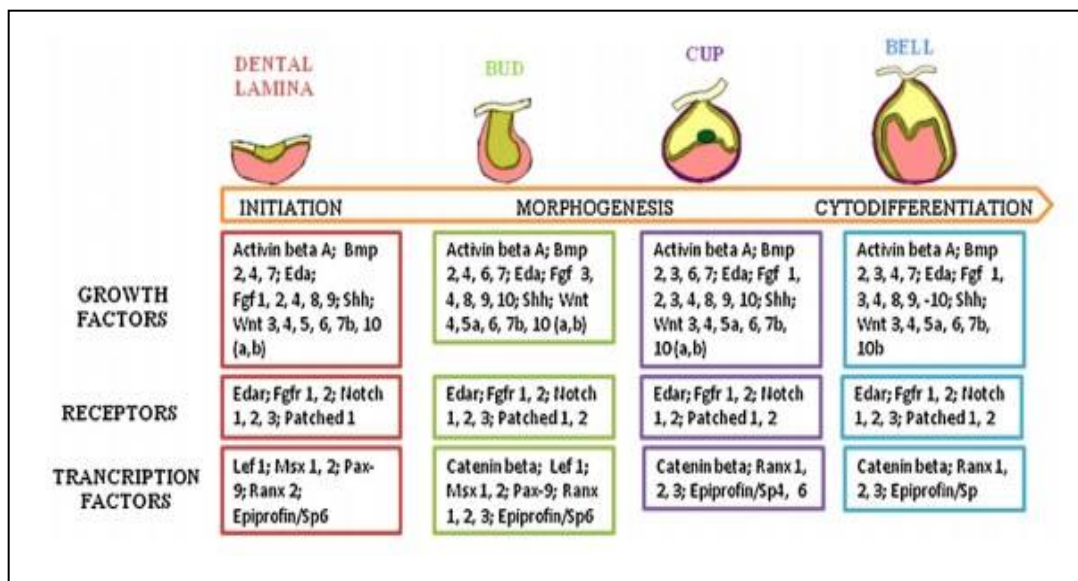
##### **4) Sonic hedgehog (Shh)**

Shh acts within the enamel bud to produce tooth growth and morphology (Dassule *et al.*, 2000).

### **5) Wnt pathways.**

The WNT protein family is involved in tooth development and is important in orchestrating an intracellular signaling pathway that involves the  $\beta$ -catenin nuclear proteins and the LEF1 transcription factor. The  $\beta$ -catenin is eventually degraded if the Wnt signaling is missing to produce a negative feedback mechanism on the signaling pathway (Chen *et al.*, 1996).

It is clear from the diagrammatic representation of the early tooth model that precise timing feedback and the interaction of multiple gene products is essential for the correct formation of the size, morphology and number of teeth (Arte, 2001). The enamel knot is considered an important anatomical site within the developing tooth germ in ensuring that the multiple signaling pathways are functioning correctly (De Coster *et al.*, 2008).

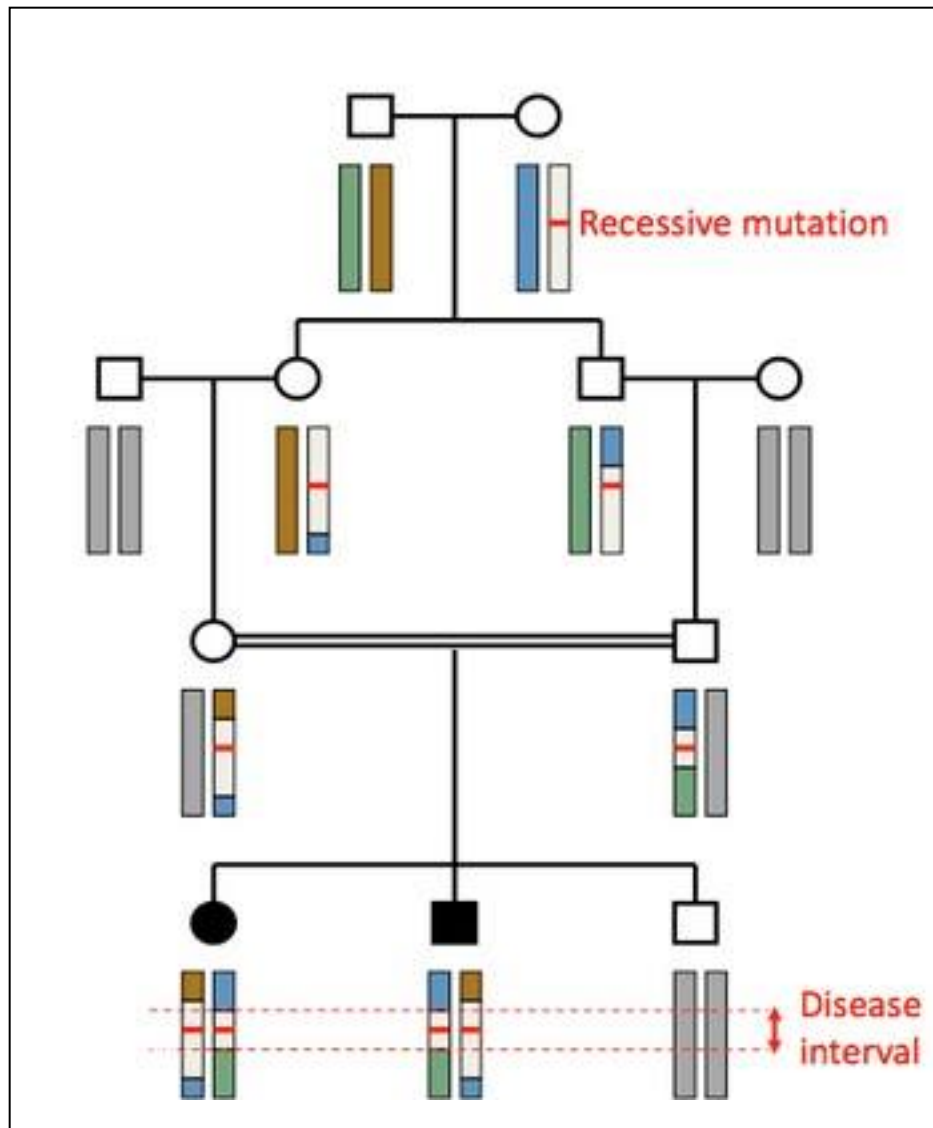


**Figure 1.4.6.1 Brook (1984) A summary of the key factors involved in tooth development genes /growth factors/receptors/transcription factors in humans and mice.**

## **1.5 Autozygosity mapping**

Individuals with a positive history of consanguinity provide an opportunity for defective gene identification. This is because affected individuals inherit both copies of the mutated gene from the common family member. Locating such chromosomal regions is known as autozygosity mapping (Mueller and Bishop, 1993).





**Figure 1.5.1 Transmission of an autosomal recessive disorder through four generations.**

The diagram shows how a consanguineous union may produce a coincidence of two recessive genes in an individual and lead to manifestation of a trait ([autozygosity.org](http://autozygosity.org))

### 1.5.1 Consanguinity

Consanguinity is defined as a union between couples who are related as second cousins or closer.

Several global communities consider a consanguineous union as socially advantageous despite the recognized associated deleterious genetic risks. The incidence and type of all recessive diseases in any given population will determine the effect of a consanguineous union. These deleterious effects are more pronounced in individuals from first cousin marriages where there was a reported increased infant mortality rate of nearly 5% compared to non-related controls (Bittles and Neel, 1994). Risks are doubled for the children of first cousin marriages.

The Birmingham Birth study found nearly 70% of British Pakistani's were related of which almost 60% were first cousins (Bundey and Alam, 1993). Compared to children from Northern European couples where the reported rate of consanguinity is less than 0.5%, the prevalence of genetic disorders was 7.9% in the children of the British Pakistani couples compared to 4.3%. Recessive disorders were ten times more common in British Pakistani children than in the European children; 3.3% and 0.28% respectively.

Access to genetic counseling and identification of at risk individuals for carrier status of recessive conditions has often been a mainstay clinical approach.

### 1.5.2 Consanguinity and hypodontia

Several isolated reports linking consanguinity with hypodontia have been reported (Philip and Caurdy, 1985; Ahmad, 1998). Most of these case studies have demonstrated an autosomal recessive mode of inheritances in a first cousin union. With hypodontia associated with various other dental anomalies (Fried, 1977; Ahmad *et al.*, 1998).

## **1.6 Methods for identifying genes behind human disease**

### **1.6.1 Functional cloning**

Cloning is an efficient method of analyzing the structure of the putative gene. This can be done in one of two ways- functional or positional cloning (Pemberton *et al.*, 2005).

Identifying the protein product of a gene is one method of assessing the activity of a gene; this is known as functional cloning of a gene (Collins, 1995).

### **1.6.2 Positional cloning**

Positional cloning identifies a gene from its chromosome location (Collins, 1995).

#### **Mapping of a disease gene**

Gene mapping involves localization of a gene by studying families when analyzing disease inheritance. This is done by identifying markers; regions of DNA that tend to be seen in family members and lie close to the gene of interest. If a marker and the gene lie close together then chances are they will stay together during the process of recombination during meiosis.

Linkage analysis is used to identify the position of known genes or genetic markers that cause genetic disease in terms of likely frequency of recombination. Diseases and markers in a family are followed and measured. Linkage analysis relies on the ability to detect recombination events between the disease and the marker during meiosis. Linkage analysis is particularly useful for the study of Mendelian traits (Öhman, 2001).

### 1.6.3 Methods of genetic testing

Although it is a valuable tool, gene mapping lacks sufficient sensitivity to detect all forms of genetic variation. Techniques including whole genome sequencing and exome sequencing have become popularized in recent times for detecting genetic variations particularly in Mendelian inherited disorders. Exome sequencing is particularly useful since we know that most Mendelian inherited disorders are transmitted on exons the coding regions of DNA and is often more cost effective (Biesecker *et al.*, 2011).

Approach	Applies to	Advantages	Disadvantages
Candidate gene	Any disease	Easy to perform for one or two genes; requires no mapping; can directly identify the causative variant/mutation	Relies heavily on current biological knowledge; success rate very low
Genetic mapping by karyotyping	Any disease	Easy to perform; no familial cases required; can detect (large) balanced events	Low resolution, only detects large chromosomal aberrations; mutation detection requires second step
Genetic mapping by linkage analysis	Inherited disease	Easy to perform	Requires large families, often identifies large loci; mutation detection requires second step
Genetic mapping by homozygosity mapping	Recessive monogenic diseases	Small families can be used	Most useful for consanguineous families; often identifies large loci; mutation detection requires second step
Genetic mapping by CNV analysis	Monogenic/monolocus disease	High resolution CNV screening; no familial cases required; can potentially identify small loci	Only investigates CNVs; cannot detect balanced events, no base-pair resolution; mutation detection requires second step
Whole exome sequencing (WES)	Any disease	Base-pair resolution exome-wide; detects most types of genomic variation; can directly identify the causative variant/mutation	Unable to detect non-coding variants; limited resolution for CNVs and other structural variation; coverage variability due to enrichment process; relatively expensive
Whole genome sequencing (WGS)	Any disease	Base-pair resolution genome-wide; detects all types of genomic variation; can directly identify the causative variant/mutation	Data analysis complex; even more expensive than exome

**Table 1.6.3.1 Mendelian disease identification approaches in Genome Biology Gilisen *et al.*,**

**2011**

#### 1.6.4 Summary of the literature

Hypodontia is a clinically challenging disorder that requires a multi-disciplinary approach to treatment. The disorder can impact on an individual's quality of life in several ways and treatment is often lengthy with lifelong maintenance, often being a burden for the individual as well as the health care provider. Birmingham has a large Pakistani population where consanguineous union is commonly practiced and looked upon as favourable from a cultural and social point of view. It has been recognized that consanguineous unions often increase the risk of an autosomal recessive disorder and this has been reported in one of the largest epidemiological studies carried out in the West Midlands. There is a definite need to recognize the genetic implications of a consanguineous union, which constitutes a complex public health issue. Hypodontia has a multifactorial aetiology although most cases are due to genetic causes. The literature supports predominantly an autosomal dominant mode of inheritance often affecting *MSX1* and *PAX9*. However an autosomal recessive mode of inheritance has been reported in a few global isolated case reports.

The aim of the present study was to examine the effect of inheritance upon hypodontia. Since genetic influences are reinforced by consanguinity, subjects with missing teeth whose parents were first cousins were examined.

I hope that by undertaking this study in a high-risk population for autosomal recessive disorders it is possible to identify a candidate gene or gene locus responsible for autosomal recessive mode of inheritance. The information imparted from this study may help educate individuals on the risk factors for this condition and help allocate resources for this disorder appropriately.

## **1.7 Aims of Present Study**

The aim of the research was to:

1. To recruit and identify cases of familial (non-syndromic) hypodontia for new autosomal recessive genes. The main criteria for selection will be an autosomal recessive pattern of inheritance for non-syndromic hypodontia.
2. To characterize these cohort of patients to determine whether these groups of patients represent a homogenous or heterogeneous group
3. To relate the clinical phenotype of potential autosomal recessive hypodontia to characteristics of known hypodontia to determine whether they correspond to a novel disorder or a known mutation
4. The long-term aim of the study would be to use autozygosity mapping in consanguineous families with hypodontia to identify disease loci and candidate genes within them. Candidate genes will be screened in the consanguineous families for pathogenic mutations.

### **1.7.1 Null Hypothesis**

The null hypothesis of the study is that there is no difference in the genotype of cases observed in a non-syndromic consanguineous population suffering from severe hypodontia with those no known history of consanguinity. This would mean the putative mutation is analogous to that determined in the general population and that a novel mutation transmitted in an autosomal fashion is unlikely.

## **1.8 Materials and Methods**

### **Patient recruitment**

Patients were recruited by free hand searching through the hypodontia clinic database Birmingham Dental Hospital. Patients with a known history of consanguinity were selected.

The first step was to find consanguineous families containing individuals affected with severe hypodontia. Subjects were then evaluated for evidence of autosomal recessive inheritance and/or gene mutations. In the West Midlands families that originated from Pakistani or Bangladeshi background have a high frequency of parental consanguinity and so, as information on the presence

or absence of parental consanguinity wasn't available in the hospital records. Patients with a Muslim family name were identified for possible further study.

#### 1.8.1 Inclusion criteria

1. Consanguineous families with one or more children affected with severe hypodontia
2. Absence of any syndrome
3. Children aged over the age of 10 years with a positive history of hypodontia.

#### 1.8.2 Consent and Ethics Approval

Professor Maher's Molecular Pathology of Human Genetic Disease Study covered ethical approval. Informed consent was obtained from all participants and the study was approved by the LREC (Local Research Ethics Committee). All clinical research adhered to principles outlined by the Declaration of Helsinki. (See Appendix for consent form)

A sample size calculation was not carried since the exact location of the putative region of the genes involved was unknown. Even though studies have reported hypodontia in a consanguineous population, the size of the studies has been too small to make findings on which to base a sample size calculation. Inclusion of more families with a single affected individual wouldn't necessarily help. It would be better if the families were larger and had more affected individuals so that the method of inheritance to be interpreted more easily.

#### 1.8.3 Clinical Assessment and of Blood Samples






10 affected family members were recruited with classical features of hypodontia. All subjects had parents who were first cousins). None of the parents were affected with hypodontia.

A full medical and dental history was taken. A pedigree analysis was constructed from the family members to accurately ascertain the affected individuals so that the pattern of inheritance could be established. In all cases radiographs were available which confirmed the diagnosis of severe hypodontia. Blood samples were taken from all affected individuals, their parents and any affected siblings and sent to the Birmingham Women's Hospital for DNA extraction.



## 1.9 Results

1.9.1 Clinical details of the 10 subjects are shown below:

<i>Key:</i>	Unaffected Male	
	Unaffected Female	
	Affected individual	
	Non-consanguineous union	
	Consanguineous union	

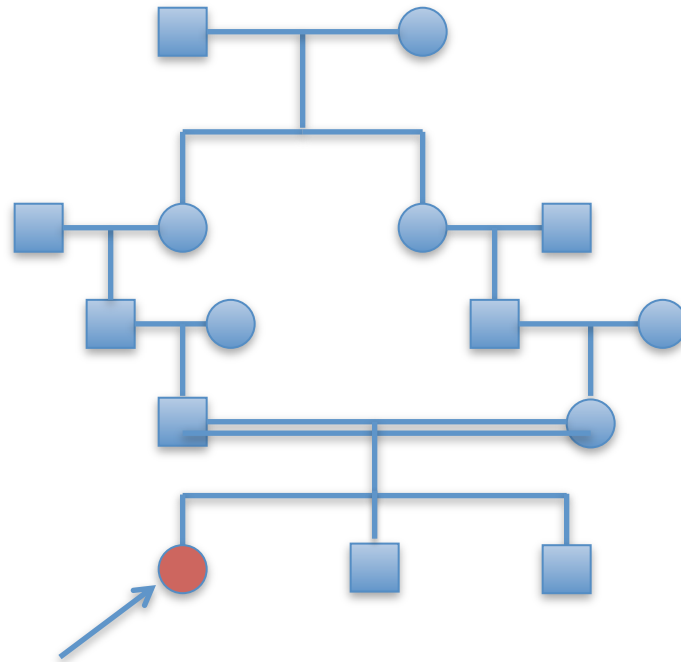
## Family 1

An 18-year old female with mild asthma presenting with a chief complaint of “gappy teeth”. The subject was previously seen at the genetics department to exclude diagnosis of X-linked hypohydrotic ectodermal dysplasia. She had increased pigmentation around the eyes. However her hair growth was normal and she had no overt temperature regulation issues. Nail growth was normal. She was one of three siblings two of whom had normal dentitions. The patient had a Class III incisor relationship with hypoplasia affecting her maxilla. There was delayed dental development in her primary dentition and an abnormal morphology associated with her upper permanent incisors. The clinical impression suggested generalized concomitant microdontia. The existing units were abnormally shaped with bulbous crowns and narrow cervical regions. The upper central incisors had dens in dente appearance, previously restored with composite restorations. There was severe alveolar atrophy in the regions demonstrating hypodontia with significant periodontal bone loss.

The following teeth were missing:

8	7	6	4	2		2	4	6	7	8			
8	7	6	5	3	2	1	1	2	3	5	6	7	8

## Pedigree Analysis



## Special Investigations

An orthopantogram highlighting 20 missing units in the permanent dentition with severe atrophy of the alveolar bone in regions of missing teeth. There is generalized microdontia with shortened dental roots. The crowns of the teeth are conical cervically.



## Family 2

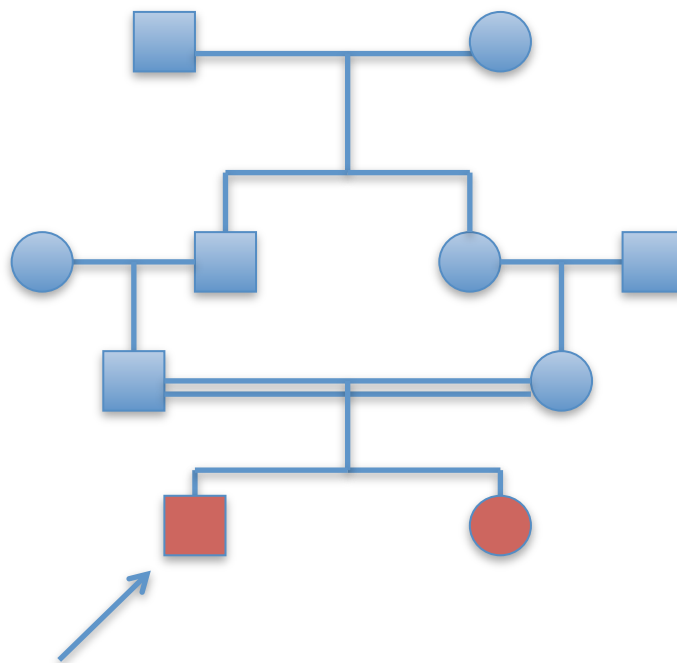
A 15-year-old male with a Class II division II incisor relationship on a mild skeletal II base with bimaxillary retrognathia.

There was no reported history of hypodontia in the primary dentition. The sibling of the proband appeared to suffer from hypodontia, although she was unavailable for interview

Teeth missing in the subject included:

8	5	2		2	3	5			
8	7	5	3	1	1	2	5	7	8

## Pedigree Analysis



## Special Investigations

An orthopantogram confirmed 13 missing permanent units

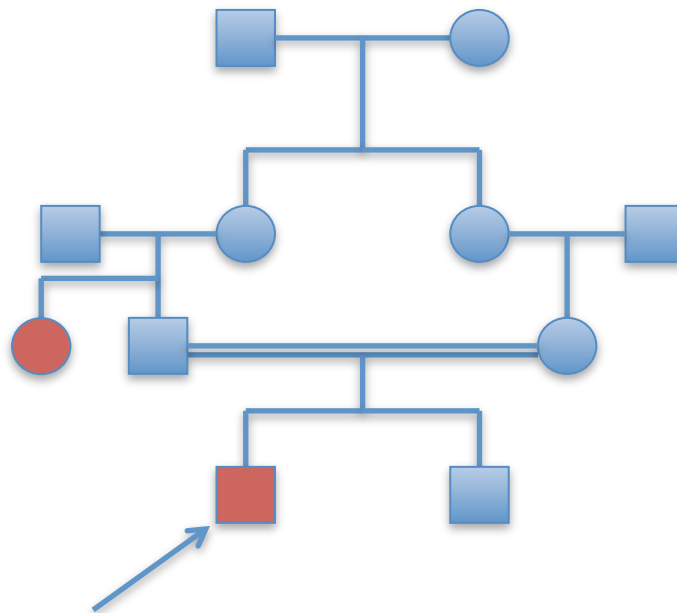


### Family 3

A 14-year old male with mild asthma and a penicillin allergy with a chief complaint of gappy teeth. There was a family history of missing teeth with the paternal aunt suffering from hypodontia. The subject had a Class II division 2 incisor relationship on a mild skeletal II base. There was a previous history of palatal ectopia associated with the upper canines. The upper central incisors had an abnormal morphology and were diminutive. The clinical examination revealed hypodontia with the following missing teeth:

8	5	4		2		2		4	5	8
8	5	4	3	1		1	3	4	5	8

### Pedigree Analysis



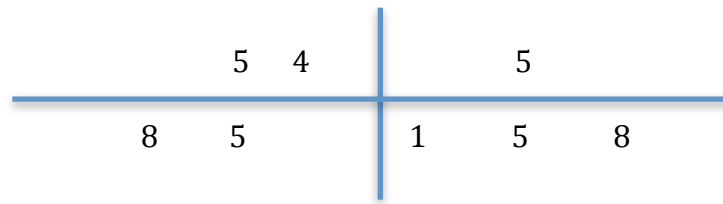
## Special Investigations

An orthopantogram highlighting 14 missing permanent units

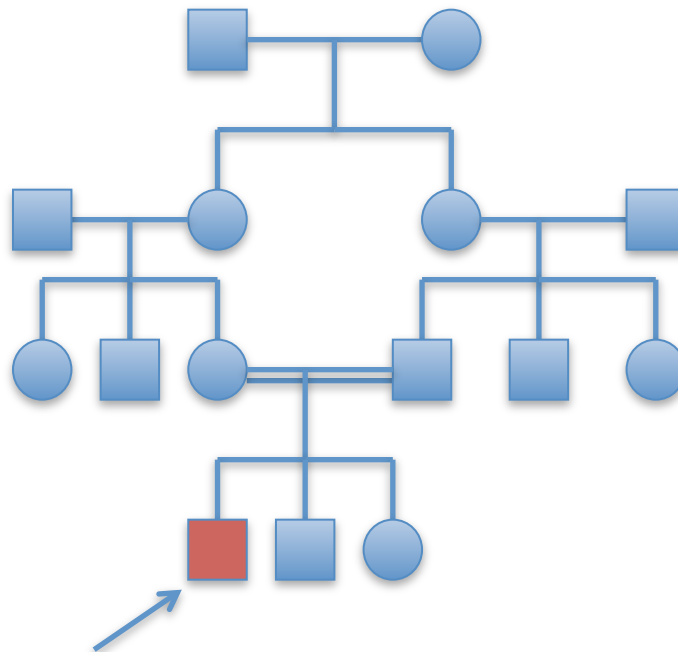


#### Family 4

A 14-year old male with no family history of hypodontia and no reported hypodontia in the primary dentition presented with a Class I malocclusion. His upper left canine was ectopic and palatal in position and the upper lateral incisors were diminutive. A radiographic examination revealed an unerupted midline supernumerary. The teeth missing were:



#### Pedigree Analysis





## Special Investigations

An orthopantogram demonstrated 6 missing permanent units

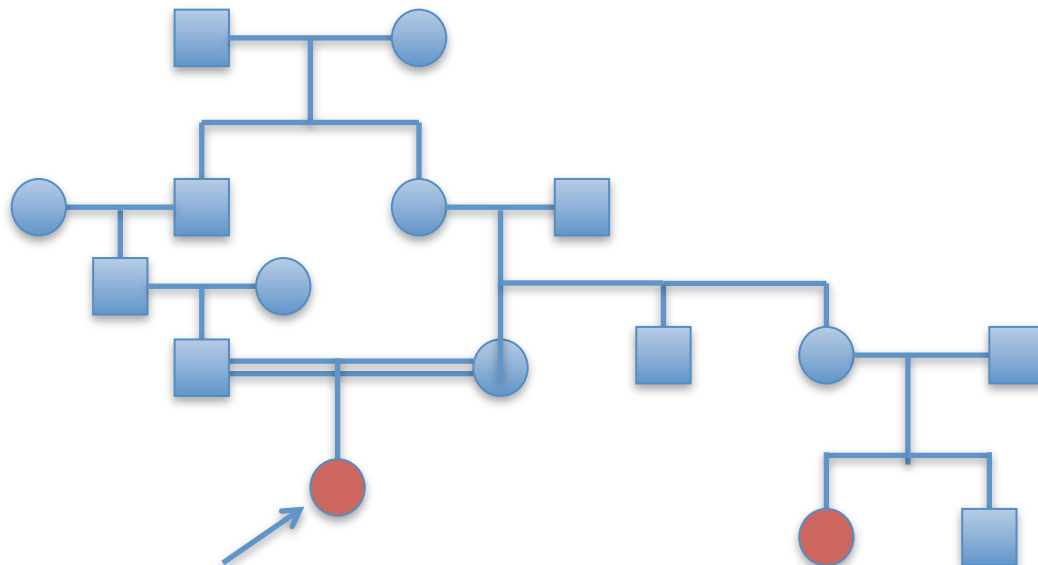


## Family 5

A 12-year-old female who had a chief complaint of gappy teeth. There was a family history of hypodontia however it was unconfirmed if the affected individual was the offspring of a consanguineous union. The subject in examination was developing a Class III skeletal relationship and delayed dental development. There was hypoplasia affected the upper right and lower left and right central incisors. The first permanent molars and incisors had abnormal morphology and the erupted incisors were conical shaped. The pulp morphology of the molars was taurodont. The missing permanent units were:

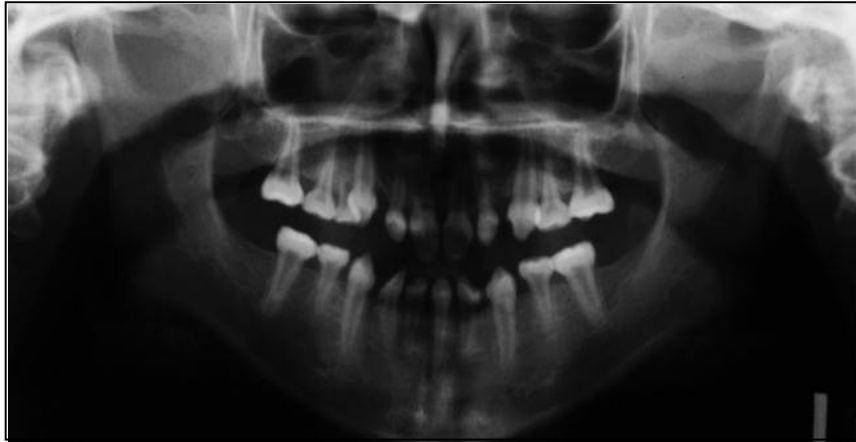
8 7 5 4	4 5 7 8
8 7 5 4	4 5 7 8

## Pedigree Analysis



## Special Investigations

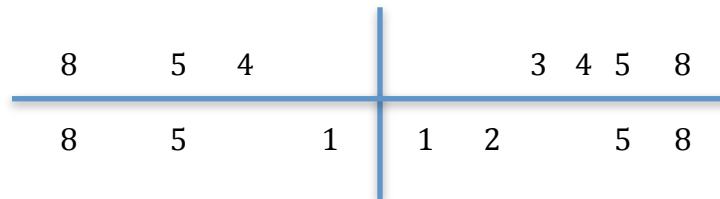
An orthopantomogram radiograph confirmed 12 missing units (excluding the third molars).



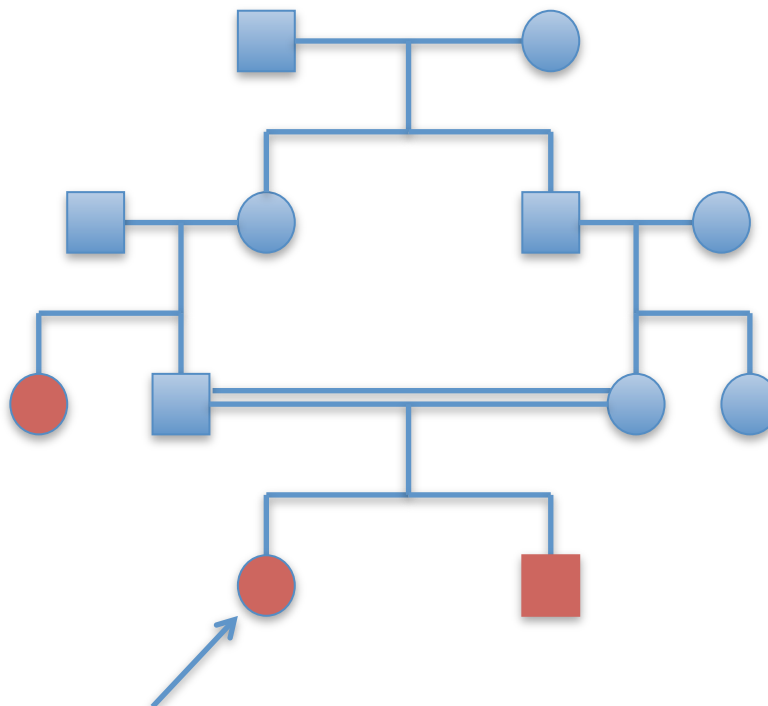
## Family 6

A 15-year-old female was dissatisfied with her gappy and small teeth. She had a family history of missing teeth. Her older brother and aunt had missing teeth. It was not possible to ascertain if the parents of the affected aunt were consanguineous. The subject had a skeletal III profile. There was infraocclusion of the lower second primary molars and the upper lateral incisors were diminutive.

The following teeth were absent:



## Pedigree Analysis



## Special Investigations

An orthopantogram confirms 10 missing permanent units.

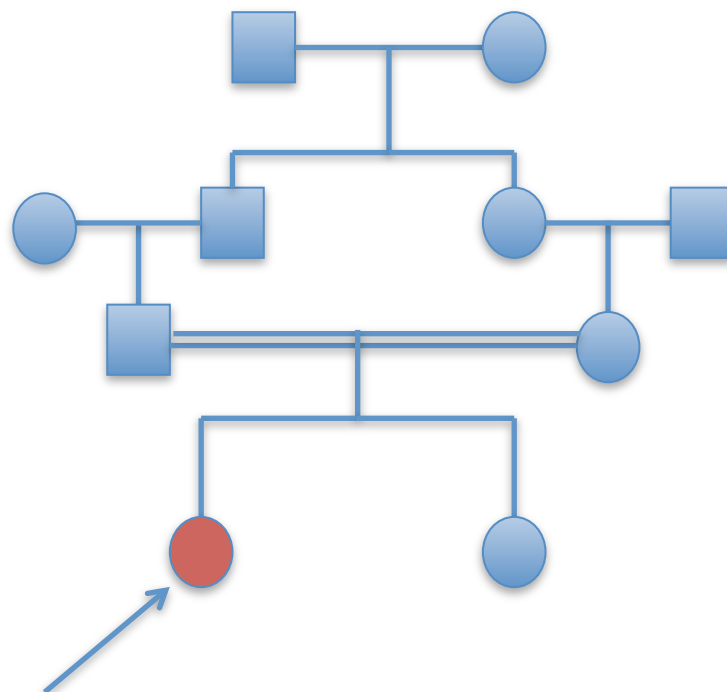


## Family 7

A 12-year old female with no family history of hypodontia had a Class I incisor relationship in the mixed dentition phase with several retained primary teeth. There was a history of both hypodontia and microdontia in the primary dentition; the lower left canine was unerupted which provides evidence for delayed dental development. The teeth missing were:

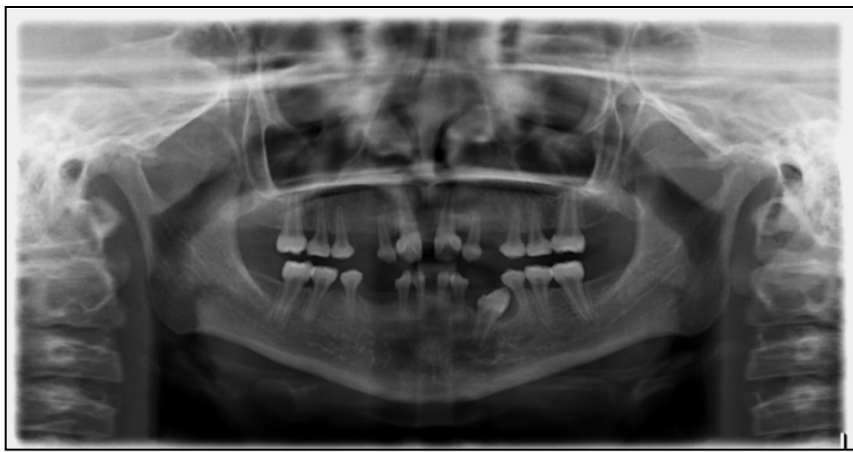
8 7	5 4 3 2		2 3 4 5	7 8
8 7	5 4 3 2	1	1 2	4 5 7 8

## Pedigree Analysis



## Special Investigations

An orthopantogram confirmed the absence 13 permanent units.



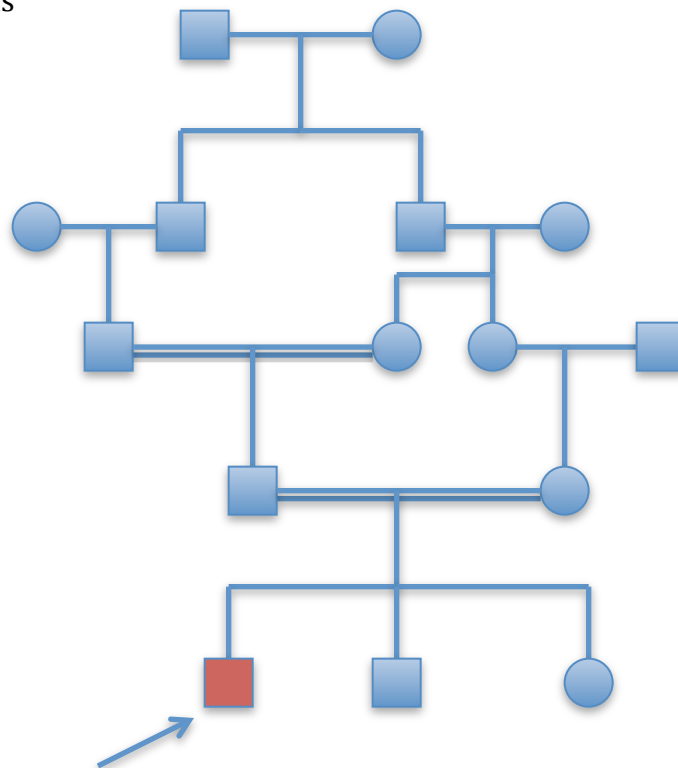
## Family 8

A 15-year-old male with late diagnosis of acute lymphoblastic leukemia concerns regarding multiple missing teeth. There was no family history of missing teeth. There was a history of hypodontia in the primary dentition and Class III malocclusion. Intra-orally he demonstrated severe hypodontia. The teeth that were present were conical and diminutive.

The teeth absent were:

8	7	6	5	4	2		2	4	5	6	7	8
8		6	5		3	1	1	3	5	6	7	8

## Pedigree Analysis





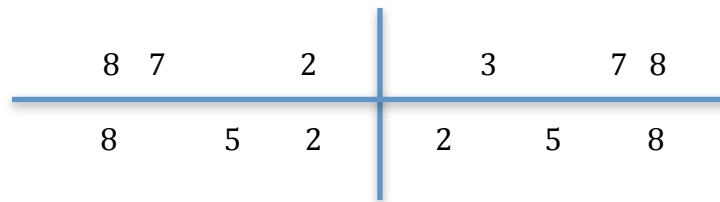
## Special Investigations

An orthopantogram confirmed 19 permanent missing units excluding third molars.

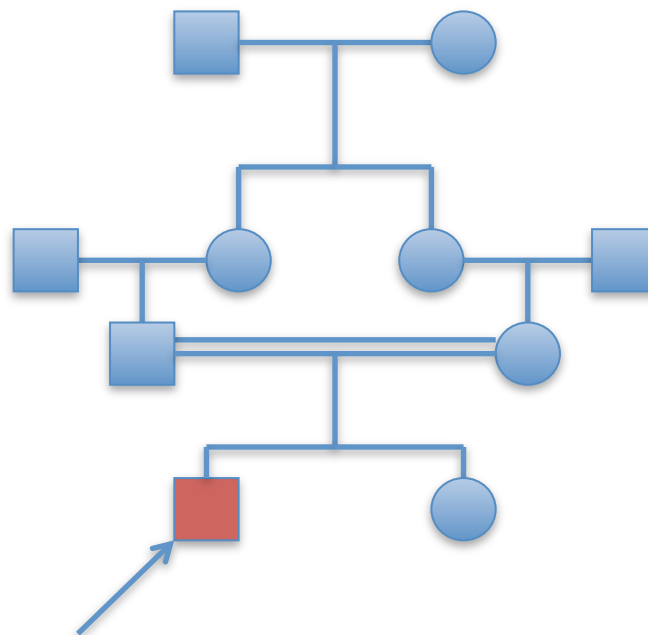


## Family 9

A 12-year old male with mild asthma was dissatisfied with his gappy teeth. There was no family history of missing teeth or hypodontia in the primary dentition. He had a Class III incisor relationship and a high caries rate. There was delayed dental development. The upper right canine was present and in a favorable position for eruption. The teeth missing were:



## Pedigree Analysis



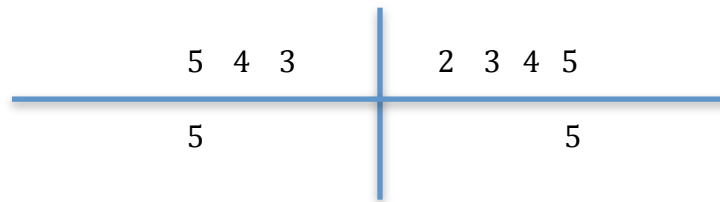
## Special Investigations

An orthopantogram confirming the absence of 8 permanent units

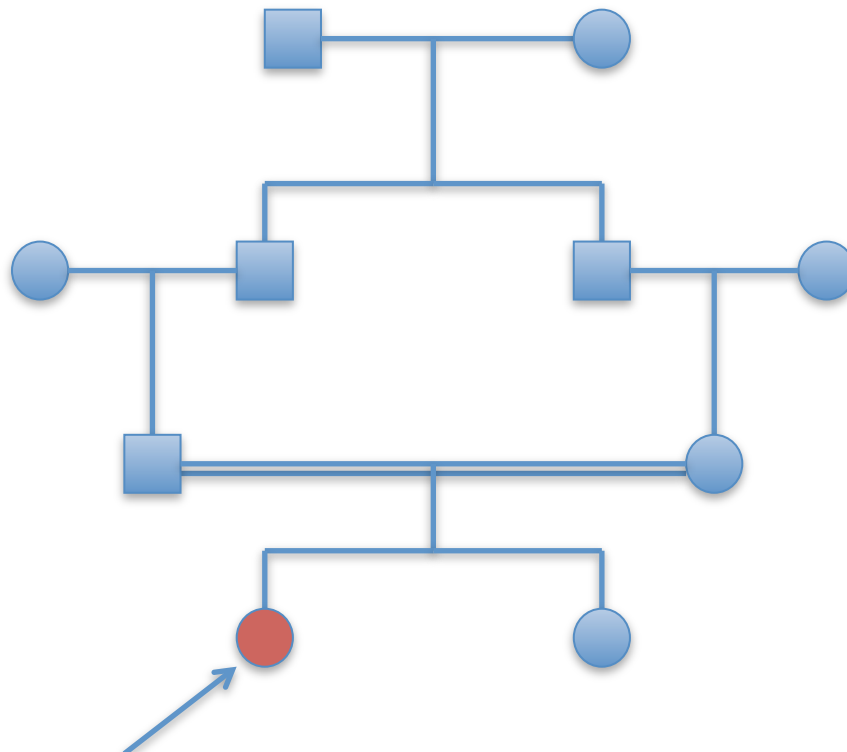


## Family 10

A 19-year old female was dissatisfied with her gappy teeth. There was a Class III incisor relationship on a mild skeletal III base. There was no history of hypodontia in the primary dentition. The upper right lateral incisor was diminutive and there were several retained primary units. The teeth missing were:

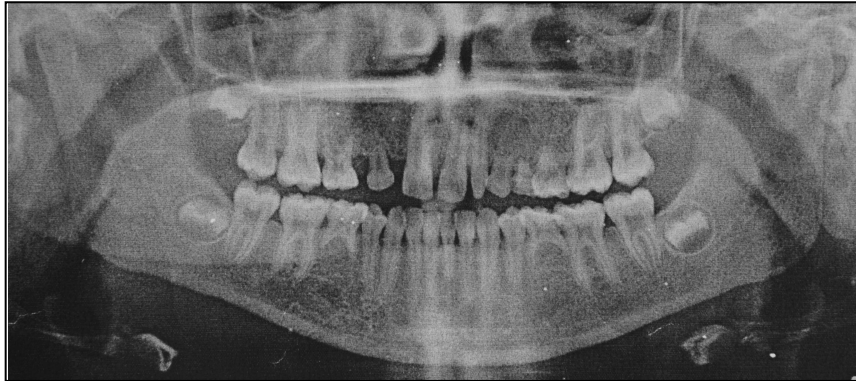


## Pedigree Analysis



## Special Investigations

An orthopantogram confirmed the absence of 9 missing permanent units excluding third molars



### 1.9.2 DNA Analysis

DNA samples in this project were extracted from peripheral blood samples. The West Midlands Regional Genetics Laboratory carried out the extraction procedures.

**Gene sequenced by Atif Al-Saedi**

#### **Polymerase Chain Reaction (PCR)**

Target DNA was amplified by using PCRs to give numerous identical copies of the target DNA sequence. Each standard PCR reaction should contain the following reagents: DNA from the patient's sample, which is the template to be sequenced, two specific oligonucleotide primers designed to be complementary to the target DNA; Taq polymerase, which is isolated from *Thermusaquaticus* to function in the replication step by step building of each single strand of the target DNA which is then cut by the primers into a new, double-stranded DNA.

#### **Primers design:**

Primers used in the study included Exon Primer and Primer3. The length of each primer should be from 18-26bp and primers were designed to amplify the coding exons of the targeted genes plus intron-exon boundaries. The amplified segment was not longer than 600bp so that the large exon should be covered with at least two pairs of primers to ensure that overlap occurred.

In order to further investigate whether the families in this study might have an inherited cause of hypodontia subjects were screened for a mutation in 3 hypodontia genes. Dr Atif Al-Saedi, 2013 (Personal Communication see Figure 2.1) No mutations were detected in *PAX9*, *MSX1* or *WNT10*. Linkage studies were undertaken for the *LTBP3* gene, which was previously identified in a consanguineous Pakistani family where oligodontia was inherited along with short stature in an autosomal-recessive fashion. A homozygous nonsense mutation was identified within *LTBP3*, the gene associated for osteoclast function. Amongst all the patients of the six families, only proband of Family 5 showed homozygosity that could be

linked to the *LTBP3* gene. Further studies would be required to identify whether or not a mutation in *LTBP3* might be present in family 5. However all examined probands had normal limb-torso ratios and head circumference.

According to these findings, it was not possible to confirm any relation of these genes in causing oligodontia. No mutations were detected in PAX9, MSX1 or WNT10A.

Gene	Transcript ID	Location	Number of exons	Mutation
PAX9	NM_006194	Chr14q13.3	4 coding exons	N/A
MSX1	NM_002448	Chr4p16.2	2 coding exons	N/A
WNT10A	NM_025216	Chr2q35	4 coding exons	N/A

**Figure 1.9.2.1 Genetic Analyses for mutations (Al-Saedi, 2013)**



- 1.9.3 Comparison of Clinical features of 10 individuals with severe hypodontia and parental consanguinity with known causes of inherited hypodontia.

### **Associated dental anomalies**

Several dental anomalies that have been reported in association with hypodontia were demonstrated in the present study including: delayed formation and eruption of teeth, ectopic eruption, microdontia and shape, altered tooth shape ectopic maxillary canines, submergence of primary molar and taurodontism (Seow and Lai, 1989; Bacetti, 1998). It is possible to compare the present findings with those reported in population studies in individuals with hypodontia.

### **Delayed dental development**

Four out of the ten cases demonstrated delayed dental development. Dental development in children with mild to moderate hypodontia has been found to be significantly delayed compared with the controls (Tunc *et al.*, 2011). Delayed formation and eruption of premolars and molars was also found in children with agenesis of the lower third molars along with other tooth types (Garn *et al.*, 1961). Children with severe hypodontia have dental developmental mean ages 1.8 years for boys and 2.0 years for girls later than their chronological ages (Rune and Sarnas, 1974). A gradient of delay in tooth formation has been described in patients with hypodontia (Garn *et al.*, 1961). Theories on the aetiology and pathogenesis of isolated hypodontia involve the process of development of teeth and their local environment. The status of a developing tooth is not independent of the adjacent teeth and the failure of one tooth germ to develop may be reflected in anomalies of others, including, delay in development (Sofaer *et al.*, 1971; Mohammed, 2000). The

present finding of a delay in the development of the teeth adjacent to the site of the agenesis support the local nature of the aetiology and pathogenesis of hypodontia.

However in severe hypodontia there is a great deal of variation in tooth formation and each case must be assessed individually.

A strong correlation exists between hypodontia in primary and permanent dentitions. Children with hypodontia in the primary dentition nearly always show hypodontia of the successional teeth (Hobkirk *et al.*, 2011). This has been demonstrated in three out of the ten present cases.

### **Reduction in tooth size and form**

Tooth shape and form demonstrated a degree of heterogeneity across the ten cases in keeping with the literature. Reduction in the mesio-distal dimensions of teeth has been reported in individuals with hypodontia (Lyngstadaas *et al.*, 1996). The relationship between moderate or severe hypodontia and generalized microdontia is well established (Yaqoob, 2011).

A gradient for tooth-size reduction with varying degrees of hypodontia has been proposed; mild hypodontia might be associated with smaller deviations from ideal tooth widths whilst in severe hypodontia the teeth that do form are much smaller than normal (Grahnen, 1956, Garn and Lewis, 1970).

The association of a peg shaped lateral incisor when the contralateral incisor is absent is shown in two the reported cases. The association of mesio-distally reduced or peg shaped lateral incisors and hypodontia has been previously cited (Arte and Pirinen, 2004). Mesio-distally small or peg-shaped lateral incisors are associated with agenesis of second premolars (Bacetti, 1998). This was apparent in all but one of the cases where there was either unilateral or bilateral agenesis of lateral incisors. The frequency and inheritance patterns of missing, peg-shaped, and small upper lateral incisors in

families suggest different expressions of one dominant autosomal gene with reduced penetrance (Forestier *et al.*, 2008).

Hypodontia and microdontia are more common in females whilst supernumerary teeth and macrodontia are more common in males suggesting that there may be a genetic linkage between dental anomalies. A multifactorial model with a continuous spectrum, related to tooth number and size, with thresholds has been suggested (Brook, 1984). Position on the scale depends upon the combination of numerous genetic and environmental factors; each with a small effect. The proportion of affected relatives varies with the severity of the condition in the probands.

### **Enamel hypoplasia, hypocalcification**

Three out of the ten subjects had enamel defects. Recessively inherited hypodontia has been reported in a large family mapped to chromosome 16 with an association between hypoplasia and hypodontia (Ahmad *et al.*, 1998). The present findings support this association since there was a positive history of reported generalized hypoplasia.

### **Ectopic maxillary canines**

Families 3 and 4 had a history of palatal canine ectopia and it is not surprising that there is a concomitant association between this anomaly and absence of the ipsilateral lateral incisor. Displaced canines and missing or peg-shaped lateral incisors are known to be associated (Becker *et al.*, 1981; Brin *et al.*, 1986). A study of orthodontic patients with at least one palatally ectopic canine showed that in a high proportion of cases the lateral incisors adjacent to the ectopic canines were missing (Zilberman, 1990).

## **Taurodontism**

Taurodont molars were seen in family 5. Investigations of patients with hypodontia and their siblings have revealed an association between taurodontism and severe hypodontia (Stenvik *et al.*, 1972; Seow and Lai, 1989). Taurodontism of lower first molars was seen in a Dutch study; 29% of oligodontia patients had taurodont molars compared with 10% of the control group (Schalkand van der Weide, 1993).

## **Shortened dental roots**

Four out of the ten cases had abnormal or short dental roots. Tooth agenesis has been reported in 46% of individuals with short roots of permanent teeth, with the maxillary central incisors and premolars most affected, in this condition, also referred to in the literature as short root anomaly (Lind *et al.*, 1972). Although short roots were not seen consistently in the present study the findings support an association.

## **Skeletal/facial traits**

Six out of the ten cases had a Class III skeletal discrepancy. In the U.K. 3-5% of subjects are Class III (Ast *et al.*, 1965). A number of studies have assessed the skeletal pattern of hypodontia patients. Some have reported Class I skeletal patterns but the majority have shown that patients with hypodontia tend to have more a retrognathic maxilla, as is seen in patients with Class III malocclusions (Wisth *et al.*, 1974). This tendency becomes more significant as the severity of the hypodontia increases, especially when more than one tooth type is missing (Acharya *et al.*, 2010).

## Dental agenesis

In eight of the ten cases the more localized type of incisor premolar hypodontia was seen. This occurs in approximately 8% of the population (Niemenen *et al.*, 1995). Following the third permanent molar the most commonly absent teeth in Europeans are the mandibular second premolars (41%) and the maxillary lateral incisors (23%). Canines, first molars and second molars are rarely absent in hypodontia (Polder *et al.*, 2004). If these teeth are missing it is usually seen in association with severe forms of syndromic hypodontia (Cobourne, 2007).

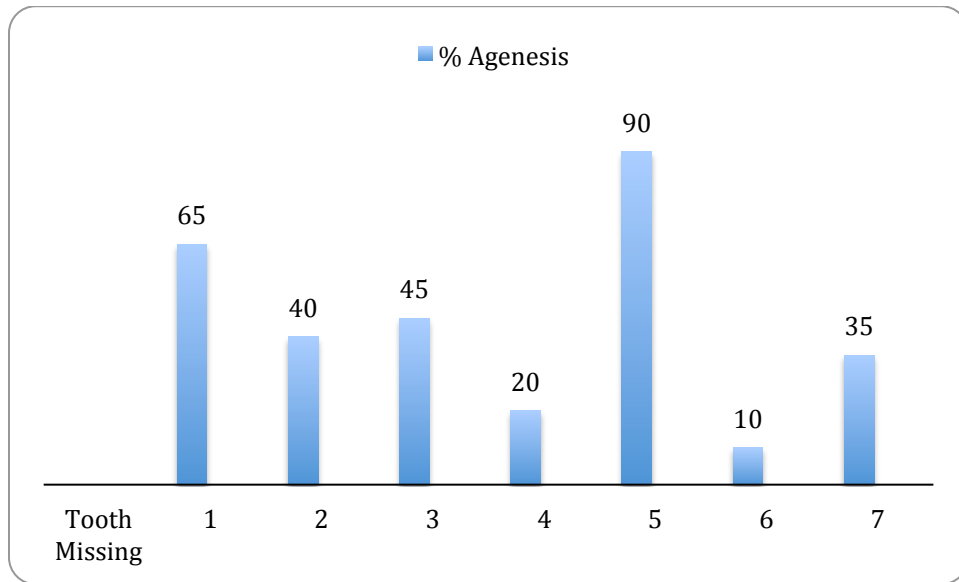
The mean number of teeth missing for all 10 subjects was 12.5. The proportion of teeth missing was evenly distributed amongst the anterior and posterior units.

In the 10 families studied there was a high proportion of bilateral missing maxillary lateral incisors. 54% of the subjects had bilateral absence of maxillary lateral incisors, which is in agreement with the literature (Polder *et al.*, 2004). The bilateral absence of lateral incisors is the only example of missing teeth with prevalence greater than 50% (Hobkirk *et al.*, 2011). Indeed it is more common for maxillary lateral incisors to be absent bilaterally and other teeth to be absent unilaterally. Canines are rarely missing; a meta-analysis has shown that the frequency of missing canines in the maxillary arch was only at 0.1% and in the mandibular arch 0.02% (Polder *et al.*, 2004).

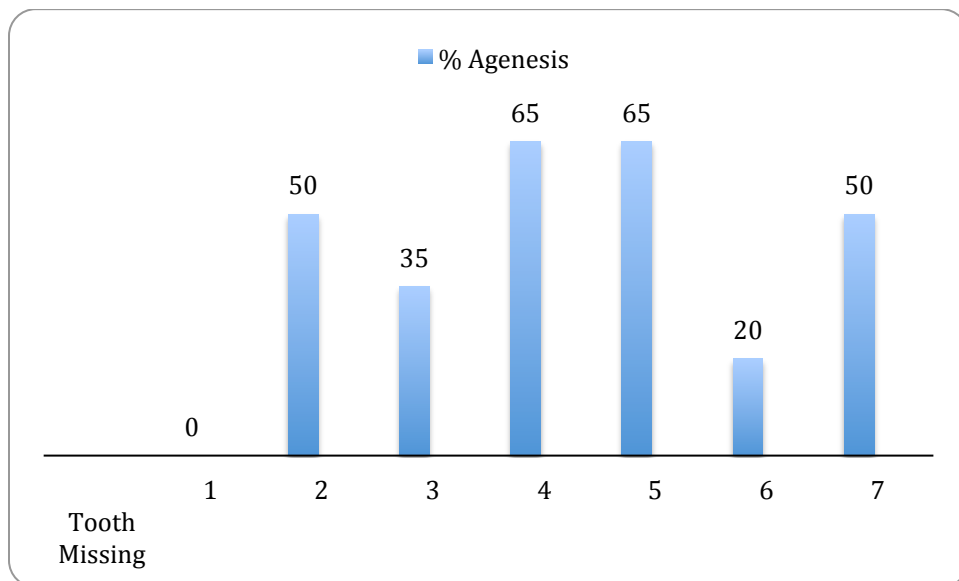
However in seven of the ten families a mixture of maxillary and mandibular canine teeth were absent a rare finding in non-syndromic hypodontia. In the maxillary arch 35% of individuals had congenital absence of canines; two subjects had bilateral absence and three subjects' unilateral absence.

Certain genes have been implicated in dental agenesis. The MSX1 gene is associated with hypodontia predominantly affecting the third molars and second premolars and PAX9 is the gene

causing isolated molar oligodontia, sometimes in combination with other missing teeth (Cobourne, 2007; Galluccio *et al.*, 2012). A non-sense mutation in AXIN2 has been reported to cause familial oligodontia with a phenotype more severe than that described for MSX1 and PAX9 (Lammi, 2004; Galluccio *et al.*, 2012). This mutation can lead to the absence of most permanent molars; premolars lower incisors and upper lateral incisors. However the upper central incisors are preserved as was found in the present study. Both MSX1 and PAX9 are dosage sensitive genes. In humans, haplo-insufficiency of MSX1 or PAX9 results in loss of function in about 50% of cases with resulting severe generalized hypodontia in MSX1 and molar agenesis in PAX9 (Galluccio *et al.*, 2012). Milder phenotypes maybe the result of a defective allele generating an aberrant protein that acts in a dominant- negative manner or has a novel function. A mutation in PAX9 has also been associated with severe hypodontia in a family affected with agenesis of most permanent molars and a variable absence of second premolars and mandibular incisors (De Coster, 2009). In the present study the agenesis appeared to consistently affect premolars, molars and mandibular incisors.



**Figure 1.9.2.1 Dental Agenesis distribution in Mandibular Arch by tooth type**



**Figure 1.9.2.2 Dental Agenesis distribution in Maxillary arch by tooth type**

## 2.0 Discussion

The present project attempted to identify individuals with autosomal recessively inherited hypodontia by preferential study of subjects with hypodontia who had consanguineous parents. Ten unrelated individuals were recruited and they underwent detailed clinical and radiological assessment. Panoramic radiographs and examination of dental chartings characterized tooth agenesis. A comprehensive family history was taken to identify possible associated anomalies and to ensure that the families suffered from true non-syndromic hypodontia. There were no remarkable extra-oral features that suggested an underlying syndrome in any subject e.g. ectodermal dysplastic features such as nails/sparse hair/absent sweat glands. Similar numbers of males and females were affected. Extra-oral features were consistent with those of individuals suffering from severe hypodontia, for example retrusive soft tissue patterns and an over-closed appearance due to a lack of teeth. All probands demonstrated more than 6 missing permanent units and an equal distribution of posterior and anterior tooth agenesis. It was not possible to determine whether each subject had an inherited form of hypodontia and, if so, to identify the mode of inheritance. Mutations are rare and there is no data on population frequency

Four out of the ten families had affected relatives, which suggests that there was an underlying genetic basis for the anomaly. In Family 2 there is an affected brother and sister and so X-linked inheritance can be excluded in this and other families with affected females. It is possible that there was an autosomal dominant mode inheritance might be operating in some families. In families in which there is only one affected individual then the lack of other affected relatives might result from either a *de novo* mutation in the proband or non-penetrance in a carrier parent. In Family 3 and 6 where an aunt and proband are affected then several relatives would have to be non-penetrant. The



families were selected because of the presence of consanguinity and so autosomal recessive inheritance would be suspected. Autozygosity mapping studies could be used to identify the genetic basis of these families and so define the exact mode of inheritance and the risk to relatives.

Non-syndromic or familial hypodontia is by far the most common form of hypodontia and has wide phenotypic can be varied. The congenital absence of six or more missing permanent teeth (excluding the third molar) is observed in 0.14% of the population and is considered to be highly inheritable (Polder, 2004; Boogard, 2012). The condition can follow an autosomal dominant, autosomal recessive or sex-linked pattern of inheritance with considerable variation in penetrance and expressivity (Cobourne, 2007). This has been clearly shown in the present study since not all families with a similar history of degree of consanguinity demonstrated similar patterns of missing teeth.

Gene mutations have an impact on agenesis patterns in different ways. PAX9 sequence alterations lead to agenesis mainly of molars whilst MSX1 mutations have been implicated primarily in congenitally missing premolars (Cobourne, 2007; Galluccio *et al.*, 2012). Severe agenesis of both molars and premolars has been noted in patients with an AXIN2 mutation (Cobourne, 2007), whereas missing incisors are the manifestation of EDA-associated non-syndromic oligodontia (Song *et al.*, 2009). The degree of hypodontia was severe in all subjects and the pattern of teeth missing did not match with reports in the literature. Agenesis of mandibular and maxillary canines and first permanent molars is rare. Many individuals with hypodontia have no identifiable mutation in any of the genes mentioned. These findings suggest that there could be other candidate genes involved in subjects demonstrating missing canine teeth that are unknown at present. Mutations in the commonly affected genes do not always marry with the phenotype seen. A study of 15 unrelated

males with non-syndromic oligodontia identified the EDA gene as a candidate gene in this condition (Song *et al.*, 2009). Several subjects had congenitally missing maxillary and mandibular canines, which is very rare in isolated oligodontia (Kim, 2006). This correlates with the phenotype seen in the present study.

The frequency of agenesis associated with maxillary and mandibular first molars is low (0.03% and 0.01%) respectively (Hobkirk *et al.*, 2012). There may be an unknown causative factor with respect to these teeth that requires further investigation. Genes encoding for growth and transcriptional factors BMP4, FGF8, DLX, TBP, which have been reported to contribute to the overall process of odontogenesis, are possible candidate genes (Gerits *et al.*, 2006). Defects in these genes could explain the mismatch between the high number of unusually missing teeth and the negligible contribution of the 4 genes that were looked at in our study. Studies have failed to identify linkage to MSX1 in five unrelated families with hypodontia (Nieminen *et al.*, 1995). No find any mutations associated with MSX1 or PAX9 in 20 Vietnamese families (Frazier-Bowers *et al.* 2002).

A meta-analysis has concluded that seven genes including PAX9, EDA, MSX1, AXIN2, EDAR- ADD, NEMO, and KRT17 in order of decreasing frequency— are currently known to have a potential for causing non-syndromic oligodontia (Ruf *et al.*, 2013).

The clinical phenotype observed in the present study correlates with that described in the literature. Concomitant dental anomalies were seen consistently, which suggests that the pathway for these presenting phenotypes is likely to be similar. Embryonic development of mammalian teeth relies on reciprocal inductive signaling from those factors responsible for determining the shape and position of the teeth including MSX1, MSX2, and PAX9 (Pemberton, 2005; De Coster, 2009; Cobourne and Sharpe, 2013). Abnormal function of these specific proteins results in anomalies in

the number the shape and structure of the developing tooth (Galluccio *et al.*, 2012; Cobourne and Sharpe, 2013).

The implications of the study to the subjects include the risk of transmitting the disorder to offspring as well as the risk factor that consanguinity may propagate the disorder. In any potential genetic disorder a sensitive approach must be taken towards patient management. Genetic counseling should be offered and consideration should be given made to any social cultural and religious practices. Hypodontia has a negative impact on a child's oral health and related quality of life. The financial consequences of an aberrant dentition have implications for the NHS as well as an on-going cost to the patient. If dental agenesis were transmitted in a dominant mode the risk of dental agenesis in children of an affected individual would be up to 50% (depending on frequency of non-penetrance). If agenesis is an autosomal recessive disorder the risk to siblings will be 25% (1 in 4) but the risk to children will be small - unless the partner of the proband carries a mutation in the relevant gene, a rare occurrence unless the partner is a relative.

### **3.0 Conclusion**

Oligodontia is a genetically heterogeneous disorder and to date there has been no identification of mutations in the genes *PAX9*, *MSX1* or *WNT10A*. Mutations in *LTBP3* were shown to be unlikely in almost all of the families in the present study. Since there were multiple family members affected in four out of the ten families, these families should be prioritised for further studies with next generation sequencing methods to identify the genetic basis of the disease (and so the exact mode of inheritance). If a new genetic cause is then identified in these families then the remaining families can then be tested for mutations in the new gene.

## 4.0 Future Studies

A recent systematic review and meta-analysis have tried identifying gene mutations associated with non-syndromic hypodontia (Ruf, 2013). Besides the previously known involvement of PAX9, MSX1, EDA, AXIN2, cases of non-syndromic oligodontia were also found to be associated with EDARADD, NEMO, and KRT17. The role of EDARADD, NEMO, and KRT17 has been reported in isolated cases of one patient each however enough cases. The list of genes in this review is still not exhaustive and will continue to grow because a number of studies where hypodontia and oligodontia were not distinguished have revealed involvement of other genes such as TGFA, WNT-10, or LTBP3 (Ruf, 2013).

Identification of a mutation for a Mendelian disease enables molecular and carrier testing in both the patient and family members and contributes to the understanding of gene functions. Traditional gene mapping approaches such as karyotyping, linkage analysis, homozygosity mapping, and copy number variation (CNV) analysis have led to great insights into Mendelian disease however they lack sensitivity. More cost-efficient sequencing strategies have been developed to study the coding part of the genome. Exome sequencing has become an important test in studying the genetic causes of Mendelian disease and whole exome sequencing could potentially identify major disease genes in at least 50% of the projects focused on rare but clinically well-defined Mendelian diseases (Biesecker *et al.*, 2011)

Exome sequencing has detected several dominant Mendelian disorders occurring “sporadically” which has previously been challenging. Genetic variants associated with these particular diseases are under strong negative selection and become rapidly eliminated from the genetic pool.

Identification of this test is important when relating to our study especially since the exact mode of transmission of agenesis is inconclusive. The lower cost and efficiency of exome sequencing is an important factor, which will increase its clinical application.

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## 6.0 Appendix



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**PRINCIPAL INVESTIGATOR:  
PROFESSOR EAMONN MAHER**

Family no:  
Patient Identification Number:

## **CONSENT FORM**





